Chapter 29
Imaging and Therapy Against Hypoxic Tumors with ^{64}Cu-ATSM

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29.1 Radiolabeled Cu-ATSM as a Hypoxia Imaging Agent for PET

In tumors, hypoxia frequently occurs due to poor vascularization and tight packing of cancer cells. Tumor hypoxia is associated with adverse prognosis due to failures in radiotherapy and chemotherapy and to tumor metastasis (Brown 1999). It is thus important to develop methods for diagnosis and therapy of hypoxic tumors. Noninvasive methods to detect hypoxic tumor have been intensively developed (Padhani et al. 2007).

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We have developed a novel positron emission tomography (PET) imaging agent, Cu-diacetyl-bis (N^4-methylthiosemicarbazone) (Cu-ATSM), which can target tumor hypoxia with over-reduced conditions. Cu-ATSM can be labeled with several Cu radioisotopes, such as ^{60}Cu, ^{62}Cu and ^{64}Cu (Dehdashti et al. 2008; Fujibayashi et al. 1997, 1999; Lewis et al. 2001; Obata et al. 2001, 2005; Yoshii et al. 2012). Cu-ATSM is reported to accumulate in hypoxic environments in many kinds of tumor cells *in vitro* (Obata et al. 2005; Lewis et al. 1999; Burgman et al. 2005). Distribution of Cu-ATSM in tumor tissues differs from that of ^{18}F-fluorodeoxyglucose (^{18}FDG), a commonly used PET imaging tracer of glucose uptake (Obata et al. 2003; Tanaka et al. 2006). Cu-ATSM shows its high uptake in regions that are hypovascular, undergoing cell cycle arrest but little necrosis, while ^{18}FDG accumulates regions of hypervascularity and cell proliferation going to necrosis (Obata et al. 2003; Tanaka et al. 2006). The mechanism of Cu-ATSM accumulation in hypoxic regions has been reported (Fujibayashi et al. 1997; Obata et al. 2001; Burgman et al. 2005; Dearling et al. 2002; Holland et al. 2009). Cu-ATSM, a rigid complex of Cu(II) and ATSM, is easily divided by reduction of Cu(II) to Cu(I) and trapped into the cells under highly reduced intracellular conditions such as hypoxia (Fujibayashi et al. 1997; Obata et al. 2001; Burgman et al. 2005; Dearling et al. 2002). Cu-ATSM rapidly diffuses into cells and tissues even in low perfusion areas and is trapped within cells under highly reduced conditions such as hypoxia (Fujibayashi et al. 1997, 1999; Obata et al. 2001; Yoshii et al. 2012; Holland et al. 2009; Bowen et al. 2011). Preclinical studies have revealed that Cu-ATSM uptake increases with higher intracellular levels of the biological reductant NAD(P)H, which is associated with hypoxia and mitochondrial dysfunction, and activity of NAD(P)H-dependent reductive enzymes, rather than oxygenic conditions (Obata et al. 2001; Yoshii et al. 2012; Holland et al. 2009; Bowen et al. 2011).

In recent years, clinical PET studies using radiolabeled Cu-ATSM have been conducted for many types of cancers throughout the world. In Japan, our institute produced a generator system of ^{62}Cu and multicenter clinical studies of ^{62}Cu-ATSM PET have been conducted using our system. These clinical studies have shown that Cu-ATSM uptake is associated with therapeutic resistance, metastatic potential, and poor prognosis in several types of cancer (Dehdashti et al. 2008; Dietz et al. 2008; Lewis et al. 2008; Sato et al. 2014; Tateishi et al. 2013). Cu-ATSM uptake is correlated with high HIF-1α expression in patients' glioma (Tateishi et al. 2013). These clinical studies have demonstrated that tumor hypoxia assessed by Cu-ATSM uptake is associated with the tumors' malignant behaviors (Dehdashti et al. 2003, 2008; Dietz et al. 2008; Grigsby et al. 2007).

29.2 ^{64}Cu-ATSM as a Theranostic Agent

^{64}Cu-ATSM can be used as a "theranostic" agent. Namely, this agent can be applied not only as a PET imaging agent but also as an internal radiotherapy agent against tumors, since ^{64}Cu shows β^+ decay (0.653 MeV, 17.4%) as well as β^- decay

(0.574 MeV, 40%) and electron capture (42.6%). The photons from electron-positron annihilation can be detected by PET, while the β^- particles and Auger electrons emitted from this nuclide can damage tumor cells (Lewis et al. 2001; Obata et al. 2005; Yoshii et al. 2011; Yoshii et al. 2016). In addition, the half-life of ^{64}Cu ($t_{1/2}$ = 12.7 h) is appropriate for both diagnostic and therapeutic use. ^{64}Cu is a practical nuclide for the use of both diagnosis and therapy, because it can be readily produced with an in-hospital small cyclotron. The therapeutic effect of ^{64}Cu-ATSM has been demonstrated in both *in vitro* (Obata et al. 2005) and *in vivo* studies (Lewis et al. 2001; Aft et al. 2003). ^{64}Cu-ATSM reduces the clonogenic survival of tumor cells under hypoxia by inducing post-mitotic apoptosis (Obata et al. 2005). This is caused by heavy damage to DNA via high-linear energy transfer (LET) Auger electrons emitted from ^{64}Cu (McMillan et al. 2015). An *in vivo* study using tumor-bearing hamsters demonstrated that ^{64}Cu-ATSM treatment increased survival time without severe toxicity (Lewis et al. 2001). These previous studies supported the feasibility of ^{64}Cu-ATSM treatment against hypoxic tumors with high-LET radiation.

29.3 ^{64}Cu-ATSM Theranostics for Cancer Stem Cells

We have demonstrated that ^{64}Cu-ATSM preferentially localizes in intratumoral regions with a high density of CD133$^+$ cells, which show characteristics of cancer stem cells or cancer stem cell-like cells (CSCs) and showed therapeutic effect against CSCs in a mouse colon carcinoma (Colon-26) and human colon carcinoma (HT-29) models (Yoshii et al. 2011; Yoshii et al. 2016; Yoshii et al. 2010). In these studies, ^{64}Cu-ATSM treatment inhibited tumor growth, and the percentage of CD133$^+$ cells and metastatic ability in ^{64}Cu-ATSM treated tumors were decreased compared to that in non-treated control tumors. ^{64}Cu-ATSM accumulated in the cells under hypoxic conditions and incorporation of ^{64}Cu-ATSM under hypoxia caused cell death in both CD133$^+$ and CD133$^-$ cells. We have demonstrated that the intratumoral ^{64}Cu-ATSM high-uptake regions exhibited upregulation of DNA repair, which results in therapeutic resistance. ^{64}Cu-ATSM high-uptake regions showed upregulation of pathways related to DNA repair along with nucleic acid incorporation (bromodeoxyuridine uptake). In addition, combination use of nucleic acid antimetabolites, such as a pyrimidine analog 5-fluorouracil, a purine analog 6-thioguanine, and a folate analog pemetrexed, enhanced the efficacy of ^{64}Cu-ATSM internal radiotherapy by inhibiting DNA repair and effectively reduced %CD133$^+$ CSCs. Therefore, our study suggested that co-administration of ^{64}Cu-ATSM and nucleic acid antimetabolites could have a potential to cure tumor malignant environment and CSCs.

29.4 Biodistribution and Dosimetry of ^{64}Cu-ATSM

We have examined biodistribution of ^{64}Cu-ATSM using mice and performed dosimetry analysis. Relatively high accumulation of ^{64}Cu was observed in the liver, small intestine, and large intestine among normal organs. ^{64}Cu were mainly excreted in the feces, but little urinary excretion was observed. Our dosimetry analysis demonstrated that the liver, ovaries, and red marrow should be considered as dose-limiting organs in ^{64}Cu-ATSM internal radiotherapy. For clinical applications, we have developed a strategy to reduce radiation doses to these critical organs while preserving tumor radiation doses by the appropriately scheduled administration of copper chelator penicillamine during ^{64}Cu-ATSM internal radiotherapy (Yoshii et al. 2014). In this method, penicillamine was orally administered at 1 h after ^{64}Cu-ATSM injection, when radioactivity was almost cleared from the blood and tumor uptake had plateaued. Using this method, penicillamine decreased ^{64}Cu accumulation in the critical organs, while maintaining tumor uptake.

29.5 This Project

Anti-VEGF antibody bevacizumab is an antiangiogenic agent in widespread clinical use for cancer. Despite the initial positive effect of this treatment, continued use of bevacizumab induces hypoxia and makes tumors malignant. Thus, additional strategies to treat the hypoxia during bevacizumab therapy are needed. In this project, we are developing a method to detect and treat tumors that became malignant by acquiring decreased vascularity and hypoxia, during antiangiogenic bevacizumab treatment, with ^{64}Cu-ATSM.

29.6 Development of a Method to Detect Vascularity and Hypoxia In Vivo

Recently, an imaging technology with single-photon emission computed tomography/positron emission tomography/computed tomography (SPECT/PET/CT) to obtain simultaneous images using two different tracers labeled with SPECT and PET nuclides with CT has been developed. By applying the SPECT/PET/CT technology, we developed a method to simultaneously visualize vascularity and hypoxia with ^{99m}Tc-labeled human serum albumin (^{99m}Tc-HSA) to detect blood pool, and ^{64}Cu-ATSM to detect hypoxia (Adachi et al. 2016). In this study, we performed in vivo imaging experiments using the VECTor SPECT/PET/CT small-animal

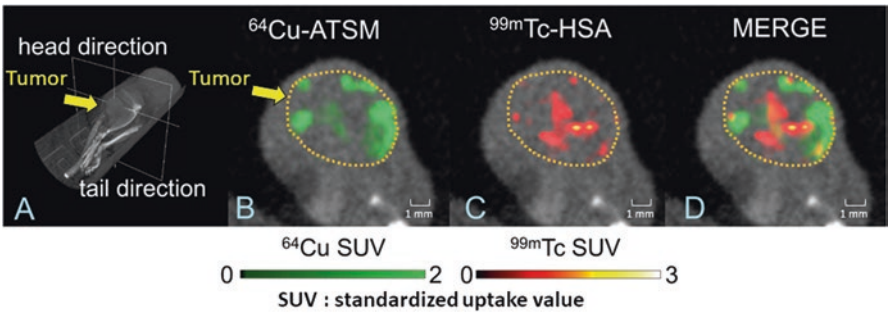

Fig. 29.1 In vivo SPECT/PET/CT imaging with ^{64}Cu-ATSM and ^{99m}Tc-HSA

scanner (MILabs) with HT-29 tumor-bearing mice. ^{64}Cu-ATSM (37 MBq) and ^{99m}Tc-HSA (18.5 MBq) were intravenously injected into a mouse at 1 h and 10 min, respectively, before scanning for 20 min. The ^{99m}Tc/^{64}Cu dual-isotope SPECT/PET/CT images were then obtained. In vivo SPECT/PET/CT imaging with ^{64}Cu-ATSM and ^{99m}Tc-HSA visualized distribution of each probe and showed that ^{64}Cu-ATSM high-uptake regions barely overlapped with ^{99m}Tc-HSA high-uptake regions within non-treated HT-29 tumors (Fig. 29.1).

To obtain a bevacizumab-treated tumor model, HT-29 tumor-bearing mice were treated with bevacizumab (5 mg/kg twice a week) for 3 weeks. Using this model, dual-isotope SPECT/PET/CT imaging with ^{99m}Tc-HSA and ^{64}Cu-ATSM was performed to check tumor vascularity and hypoxia. For comparison, un-treated tumors that showed similar size to bevacizumab-treated tumor model, were used. From imaging study, bevacizumab-treated tumors showed reduced vascularity and increased proportion of hypoxic areas within tumors.

29.7 ^{64}Cu-ATSM Therapy

For treatment study, ^{64}Cu-ATSM (37 MBq) or saline was intravenously injected into mice with bevacizumab-treated mice (bevacizumab+^{64}Cu-ATSM or bevacizumab group). For comparison, a group without bevacizumab-treatment (^{64}Cu-ATSM alone) and un-treated control were also examined. Bevacizumab+^{64}Cu-ATSM group showed the greater inhibition of tumor growth, compared with bevacizumab group, ^{64}Cu-ATSM alone group, and un-treated control, without side effect. Therefore, our data demonstrated that ^{64}Cu-ATSM therapy effectively inhibited tumor growth in bevacizumab-treated HT-29 tumors. ^{64}Cu-ATSM therapy could be a novel approach as an add-on to antiangiogenic therapy with bevacizumab (Fig. 29.2).

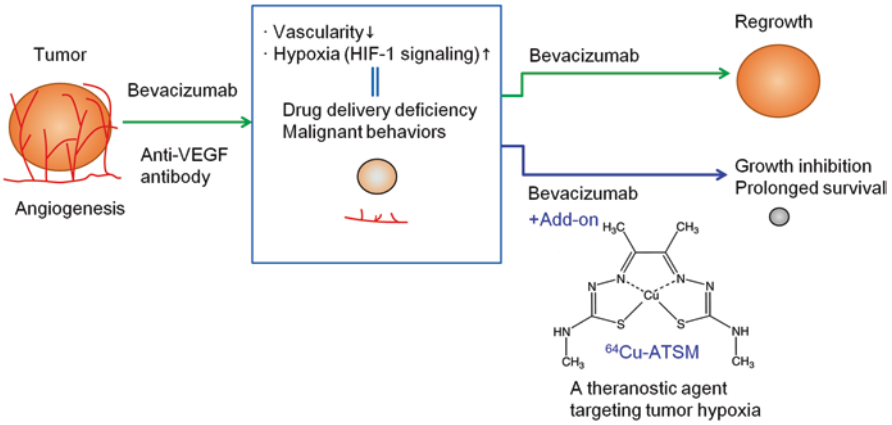

Fig. 29.2 ^{64}Cu-ATSM therapy as an add-on to antiangiogenic therapy with bevacizumab

29.8 Conclusion

We have seen that ^{64}Cu-ATSM is a promising theranostic agent targeting tumor hypoxia, which is related to tumor malignant behaviors, such as therapy resistance, metastatic potential, existence of cancer stem cells. ^{64}Cu-ATSM has unique characteristics to target tumor malignant behaviors. Therefore, ^{64}Cu-ATSM would be useful to cure malignant tumors due to hypoxia.

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